THE MORPHOLOGY AND LIFE CYCLE OF THE TREMATODE HIMASTHLA QUISSETENSIS (MILLER AND NORTHUP, 1926)

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The trematodes of the family Echinostomatidæ comprise a large number of genera which infest the alimentary tract of birds and mammals. The family and several genera were characterized by Dietz (1910). In a preliminary paper, Dietz (1909) had erected the genus *Himasthla*, with *H. rhigedana* as the type species, and in it he included *H. alincia* Dietz, *H. leptosoma* (Creplin), *H. militaris* (Rudolphi), *H. elongata* (Mehlis), and *H. secunda* (Nicoll). Linton (1928) described specimens which he identified as *H. elongata* (Mehlis), and others which he named *H. incisa* new species. A new human parasite, *H. muehlensi*, was described by Vogel (1933), who included a tabular description of all known species of *Himasthla*. Palombi (1934) described the metacercaria of a new species, *H. ambigua*, from the gills of *Tapes decussatus*.

The first complete life history of an echinostome to be recorded was that of *Echinostoma revolutum* by Johnson (1920). Subsequent life cycles, demonstrated experimentally, include that of *Hypoderæum conoideum* (Bloch) by Mathias (1925), of *Echinoparyphium recurvatum* (von Linstow) by Mathias (1927), of *Echinoparyphium aconiatum* Dietz by Riech (1927), of *Euparyphium murinum* Tubangui by Tubangui (1932), of *Euparyphium ilocanum* (Garrison) by Tubangui and Pasco (1933), of *Euparyphium malayanum* by Rao (1933), of *Echinoparyphium recurvatum* by Rašín (1933), of *Nephrostomum ramosum* by Azim (1934), of *Echinostoma coalitum* by Krull (1935), and of *Echinostoma revolutum* by Beaver (1937). The monograph of Beaver contains a review and analysis of the earlier papers. He noted certain discrepancies in the account of Johnson (1920) and suggested that the observations were made on material of more than one species. Indeed, the older descriptions are so incomplete and so inaccurate that it is exceedingly difficult to determine how many times one species has been redescribed or how many species have been confused. This condition is particularly prominent and perplexing when both adult and larval stages are considered together. Referring to descriptions of a group of eleven species of echinostome cercariæ, Beaver, p. 17, stated, "While it is not certain that they all are identical, it is certain that they can not be distinguished from each other." He pointed out that *Echinostoma revolutum* is cosmopolitan in geographic distribution and has little specificity in any of its parasitic stages. Using biometric methods on specimens reared under known, controlled conditions, he measured the normal variation in this species, noting especially the modifications induced in genetically similar material as a result of development in different avian and mammalian hosts. In the light of the more complete and precise knowledge concerning *E. revolutum*, Beaver made a critical examination of the descriptions of related species. Eight of them were reduced to synonymy and eight others were listed as of doubtful standing.

Other important contributions to the life history of these parasites include those of Ciurea (1920) and the reports of several Japanese investigators whose accounts, published in Japanese, are unfortunately not readily available. In the life cycles described, the cercariæ have been found to encyst in various mollusks, fishes and tadpoles.

Although the life history has not been completely demonstrated previously for any other species of *Himasthla*, there have been certain important and valuable contributions. Villot (1879) redescribed *H. leptosoma* (Creplin) and identified an encysted metacercaria from *Scrobicularia tenuis* as a stage in the life cycle of the worm. He traced the successive stages of development from the encysted metacercaria to the mature adult in the intestine of *Tringa variabilis*. Nicoll (1906*a*, 1906*b*) showed the close morphological agreement between an echinostome metacercaria, which he found encysted in *Cardium edule*, *Mytilus edulis* and *Mactra stultorum*, and an adult which he described as *Echinostomum secundum*. Lebour (1908) confirmed the account of Nicoll and reported an echinostome cercaria with twenty-nine cephalic spines which she believed to be the larva of *E. secundum*. Experimental infection under controlled conditions was not secured.

The life cycle of an echinostome cercaria which occurs in *Nassa obsoleta* at Woods Hole, Massachusetts, has been experimentally traced and a preliminary note was published, Stunkard (1934*a*). A more extended account was presented (Stunkard, 1937). There is adequate evidence that the cercaria is identical with the one described by Miller and Northup (1926) as *Cercaria quissetensis* and the adult is specifically identical with certain of the worms identified by Linton (1928) as *Himasthla elongata*. Since the sexually mature specimens are specifically distinct from *H. elongata*, the new combination *Himasthla quissetensis* was adopted.

To be convincing, life history studies must be carried on under well controlled conditions. The hosts used for experimental infections must be free from a previous infection which could be confused with the experimental one, or the course of development of the parasite must be followed at such short intervals that the organism can be identified at every stage. In the present study, the gulls and terns used as final hosts were removed from the nesting grounds as soon as they were hatched and, since they had never been fed by their parents, were known to be free from infection. They were fed on fish which contained no trematode larvæ, so the experimental infection was not complicated. Rats used in the experiments were laboratory raised and harbored no trematode parasites. The cercariæ of *H. quissetensis* were observed to penetrate into the gills and other organs of mollusks and encyst there. These encysted metacercariæ were fed to rats, terns and gulls, all of which were known to be free of trematode infection. The metacercariæ are infective for the final host soon after encystment and undergo little development in their cysts. It appears, therefore, that the cyst serves as a protective device to carry the larva through the acid digestion of the stomach, and that the metacercaria emerges in the small intestine of the bird or mammal. Successive stages of development were recovered from the intestine of experimentally infected birds.

The Sexual Stage in the Life Cycle

The Adult

About two hundred worms (Fig. 8) were recovered from the intestine of a herring gull, *Larus argentatus*, thirty-one days after the ingestion of experimentally infected mollusks. The shape of the body is portrayed in the figure. It is capable of much elongation and contraction and the appearance of the worms and their internal organs is modified accordingly. All of the specimens contain eggs and although the terminal portion of the uterus is usually empty, many eggs were being passed in the feces of the bird. It is possible that the region of the body immediately behind the cirrus sac would have been slightly wider if the worms had been older or more completely matured. When alive and greatly extended the sides are smooth but in all well-fixed specimens the contraction of circular and longitudinal muscles gives the edges of the body a crenated or ringed appearance. Fixed and stained specimens measure from 5 to 10.2 mm. in length and 0.5 to 0.75 mm. in greatest width. The anterior end bears a reniform collar, interrupted ventrally, and at this level the body measures about 0.3 mm. in diameter. On the collar there are thirty-one cephalic spines, arranged in a single row except for the lateral corners where two spines are situated between and behind the others. The spines measure from 0.045 to 0.058 mm. in length and 0.014 to 0.020 mm. in width. The corner spines are only slightly shorter than those in the row. The size and arrangement of the cephalic spines agree with the description and figure of these structures on the worms identified as *H. elongata* by Linton (1928). In the preacetabular region the cuticula is beset with flattened, scalelike spines which are largest near the anterior end and become progressively smaller posteriorly. The acetabulum measures 0.2 to 0.4 mm. in diameter and it is situated about the same distance behind the oral sucker. In a specimen 7.8 mm. long, the acetabulum is 0.36 mm. in diameter.

Digestive System.—The oral sucker is subterminal, spherical to ovoid in shape, and measures from 0.07 to 0.125 mm. in length by 0.1 to 0.135 mm. in width. The prepharynx is short and the pharynx measures from 0.1 to 0.13 mm. in length by 0.06 to 0.09 mm. in width. The esophagus extends to the level of the anterior margin of the acetabulum and the ceca to the posterior region of the body.

Male Genital System.—The testes are elongate, faintly lobed structures, situated in the caudal third of the body. The anterior testis measures 0.5 to 1 mm. in length and 0.25 to 0.33 mm. in width. The posterior testis is usually somewhat longer and measures from 0.7 to 1.1 mm. in length and 0.2 to 0.26 mm. in width. The testes are close together in small or contracted specimens but in larger or extended ones they are separated by a distinct interval. The sperm ducts are very small and could not be observed in whole mounts. They were traced in one series of sections. A vas deferens arises from the anterior, ventral surface of each testis and passes forward, median and ventral to the cecum, the duct from the anterior testis on the left and that from the posterior testis on the right side of the body. Both open into the caudal end of the cirrus sac where they discharge into a large, coiled, seminal vesicle which occupies the posterior third to half of the cirrus sac. The cirrus sac is long and the postacetabular portion winds about on the dorsal side of the body. Its extent is indicated in the figure but measurements of length in an organ of this shape are not significant. The cirrus is armed with small recurved spines. The genital pore is median, at the anterior border of the acetabulum.

Female Genital System.—The ovary is spherical to oval, usually broader than long, 0.1 to 0.22 mm. in diameter. It is situated near the median line, a short distance in front of the cephalic testis. The oviduct arises at the caudal end and passes backward to the oötype which is surrounded by the cells of Mehlis' gland. From the oötype, Laurer's canal proceeds in a sinuous course to the dorsal surface. The vitellaria are lateral to the intestinal ceca and extend from a level slightly in front of the caudal end of the cirrus sac to the posterior end of the body. In the fields on either side of the testes, ordinarily there are no vitelline follicles and a duct connects the separated portions of the glands. In one specimen a few small follicles were present on one side of the posterior testis. There is a cluster of follicles on either side at the level between the testes. Immediately in front of the cephalic testis, ducts arise from the ventral sides of the vitellaria and increase in size as they pass dorsally and medially where they unite to form a short common duct which discharges into the oötype. There is no seminal receptacle, but the initial portion of the uterus is filled with spermatozoa. The uterus extends in a sinuous course, backward to the level of the cephalic testis and then forward to the metraterm which lies below the caudal end of the cirrus sac. Both metraterm and cirrus sac pass on the dorsal side of the acetabulum. The eggs are large, oval, operculate, thin-shelled, and measure from 0.1 to 0.125 mm. in length by 0.06 to 0.08 mm. in width. They do not develop in the worm and those in the terminal part of the uterus contained only a fertilized ovum and masses of vitelline cells. Indeed, the terminal half of the uterus contains very few eggs, and it appears that they are passed rapidly through this portion of the organ.

The Asexual Stages

The cercariæ develop in rediæ which occupy the interlobular lymph spaces in the digestive gland of *Nassa obsoleta*. Experimental infection of the snail was not secured as the work was done during the summer at the Marine Biological Laboratory, Woods Hole, Massachusetts, and development of the eggs was so slow that hatching was not obtained before the end of the season. Johnson (1920) and Beaver (1937) reported that eggs of *Echinostoma revolutum* hatch in three to four weeks after they are passed in the feces. In *Euparyphium ilocanum*, according to Tubangui and Pasco (1933), the eggs require from two to five or six weeks to complete their development. Rašín (1933) found that eggs of *Echinoparyphium recurvatum* require two weeks at 25° and three weeks at 20° for the development of the miracidium. In all echinostome species in which development has been studied, the eggs contain unsegmented ova when they are passed. From two to five weeks, depending on the temperature, are required for development of the miracidium which then emerges from the shell and penetrates into the snail which serves as the first intermediate host. The development of all members of the family Echinostomatidæ undoubtedly follows the same general plan and accordingly it is probable that the miracidia of *H. quissetensis* complete their development in the eggs, emerge and penetrate into *Nassa obsoleta*. For *E. revolutum*, Johnson (1920) postulated that the mother rediæ were derived by metamorphosis from miracidia. Later studies on other echinostomes, however, indicate that in this family as in other digenetic trematodes, the miracidium metamorphoses into a sporocyst and the next generation is produced in the sporocyst. It is, of course, possible that there may be only a single redia in the sporocyst as reported by Linton (1914) for *Parorchis avitus* and by Stunkard (1934*b*) for *Typhlocœlum cymbium*. In the latter cases, however, the mother redia is well developed in the miracidium.

In natural infections of *Nassa obsoleta*, the interlobular lymph spaces in the digestive gland are filled with developing rediæ and cercariæ. Often the gland is much atrophied and the parasites are present in enormous numbers. In a heavy infection there may be thousands of large rediæ, filled with developing cercariæ. In addition there may be hundreds of small rediæ, often not more than one-half the size of a mature cercaria, free in the tissues of the snail. Their number is too large to believe that they have been formed in a primary sporocyst and, since they are of different sizes, they must have been produced more or less continuously. Thousands of rediæ have been taken from crushed snails and dissected under a binocular microscope but none was ever found in which young rediæ could be positively identified. It is impossible to determine whether the germinal masses in a redia are developing rediæ or cercariæ, and in all cases where development had proceeded to such a stage that positive identification could be made, the larvæ were cercariæ. The relative numbers of rediæ of different sizes indicated strongly the existence of two generations of rediæ but dissections gave no certain evidence of mother rediæ.

In order to discover the source of the small rediæ, a well-infected snail was cut in serial sections. Large numbers of small rediæ were found in the tissues and others were found (Fig. 1) within the body of mother rediæ. The identification is unmistakable. The pharynx of a redia can be distinguished from the oral sucker of a cercaria by the number and arrangement of the nuclei. The feet of the daughter redia are clearly visible, and there is no oral sucker or acetabulum. An intestine is present, although empty and collapsed. Behind the intestine there is a cleft which contains germinal cells and small germ balls, young stages of the next or cercarial generation. In addition to a daughter redia, each mother redia contained from six to sixteen other germinal masses but they were undifferentiated and their appearance gave no indication concerning their nature. It would be impossible to determine whether they were young rediæ or cercariæ if it were not for the presence of the daughter redia in the same mother redia. This discovery explains the difficulty concerning the source of the small rediæ in the tissues. The daughter rediæ are produced singly and emerge from their parental generation at a relatively early stage, before they can be distinguished readily and with certainty from developing cercariæ. There is no evidence to suggest that both rediæ and cercariæ are produced in the same redia.

The Redial Generations

In early stages mother and daughter rediæ do not differ essentially from each other in size, shape, or general appearance. The two generations can be distinguished only by their progeny and by the changes which the development of the filial generation produces in them. The mother rediæ produce daughter rediæ which mature singly, whereas the daughter rediæ produce cercariæ which mature in numbers, and when filled with cercariæ the daughter redia is much distended. Mature mother rediæ (Fig. 1) in the tissue of the snail measure from 0.5 to 0.7 mm. in length and 0.14 to 0.2 mm. in width. The pharynx measures 0.05 to 0.06 mm. in diameter.

The smallest daughter redia which was sufficiently developed to be recognized is the one found in a mother redia (Fig. 1). It measures 0.13 mm. in length and 0.056 mm. in width. The pharynx is 0.033 mm. in diameter. There are both anterior and posterior locomotor appendages or "feet." Older daughter rediæ (Fig. 2), which have emerged from their parental generations and are actively migrating in the tissues of the snail, measure from 0.2 to 0.3 mm. in length and 0.1 to 0.14 mm. in width. At this stage the pharynx measures approximately 0.04 mm. in diameter, the intestine is filled with food material and extends to the level of the posterior locomotor appendages. Around the posterior part of the intestine there is a body cavity which contains germinal cells. As the rediæ mature, the body wall becomes filled with orange pigment, the intestine and anterior locomotor appendages remain small, while the caudal region of the body and posterior locomotor appendages increase in size. The body cavity enlarges, becomes filled with cercariæ in various stages of development (Fig. 3), and the birth pore becomes functional. The redia is motile and the body may assume different shapes. It may contract to a short, cylindrical form in which the posterior locomotor appendages are hardly visible, or it may extend to a long vermiform shape in which the caudal end and posterior locomotor appendages form a tripodal support for the forward protrusion of the body. The anterior end is especially mobile. In front of the pharynx there is an active oral funnel or collar. It may form a small protuberance, about one-half as wide as the rest of the body, which disappears on retraction. Mature daughter rediæ (Fig. 3) measure from 0.8 to 1.6 mm. in length and from 0.1 to 0.25 mm. in width. The pharynx is 0.05 to 0.065 mm. in diameter.

The Cercaria

During the past seven seasons over ten thousand specimens of *Nassa obsoleta* have been examined for larval trematodes. They were isolated for forty-eight hours to find the specimens from which cercariæ were emerging. About 1 per cent of the snails was infected with *Cercaria quissetensis* although cercariæ emerged spontaneously from only about one-half of these specimens. The other infections were discovered on crushing and examining the snails. Four snails were infected with both *C. quissetensis* and *C. lintoni.*

After emergence from the snail the cercaria of *Himasthla quissetensis* swims actively for six to twelve hours. In swimming, the body is bent ventrally until it is almost spherical; the tail is extended and lashes vigorously. The swimming movement causes the cercariæ to rise in the water and during the period of active swimming they are uniformly distributed throughout a large container. Occasionally, for a few seconds the tail may cease to beat while the larva elongates and manifests a serpentine movement. When the tail is quiet, the larva slowly sinks. After six to ten hours, the larvæ do not rise high in the water

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Abbreviations

ac acetabulum
al anterior locomotor appendage
cd excretory collecting duct
cs cirrus sac
dr daughter redia
gm germinal mass
in intestine
os oral sucker
ov ovary
ph pharynx
pl posterior locomotor appendage
ts testis
ut uterus
vt vitellaria

FIG. 1. Section of mother redia, showing daughter redia and germinal masses The daughter redia is 0.13 mm. long.

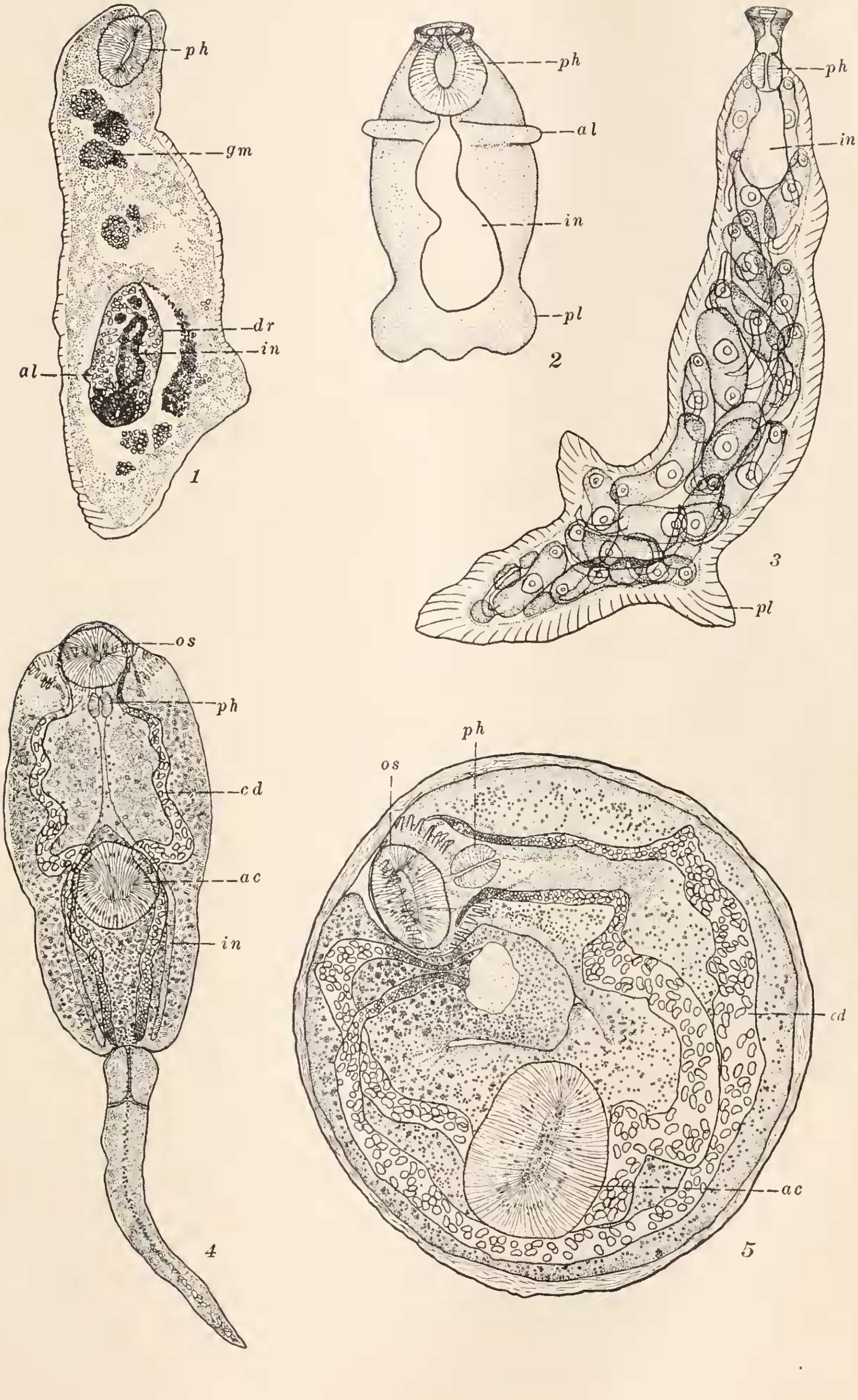
FIG. 2. Daughter redia after emergence from mother redia; drawn from a living specimen. Daughter redia is 0.2 mm. long.

FIG. 3. Mature daughter redia filled with cercariæ; drawn from a living specimen

FIG. 4. Cercaria; drawn from a fixed and stained specimen.

FIG. 5. Metacercaria; drawn from a living specimen.

Plate I



and either swim near the bottom or creep by use of the suckers. When creeping, the tail is quiet. A report on the behavior of the cercariæ in normal and dilute sea water was made by Stunkard and Shaw (1931). After twenty-four hours in sea water there is a tendency for the cercariæ to encyst. If they do not encyst, they die in about thirty-six hours.

Fully developed, naturally emerged cercariæ (Fig. 4) are elongate oval in outline. When extended the body is very narrow and when contracted the anterior end is much widened. The body measures from 0.28 to 0.65 mm. in length and 0.06 to 0.15 mm. in width. When the larva is extended, the oral sucker is protruded, and when retracted, the anterior end of the body is flattened. On the anterior portion of the body and on the tail there are small papillæ, each of which bears a delicate, bristle-like process. The base of the tail is slightly subterminal and may be either wide or narrower than the posterior tip of the body. Contracted, the tail measures about 0.17 mm. in length and 0.048 mm. in width at the base. It is almost round in cross-section and gradually tapers to the tip. In swimming, the tail extends two to three times its length when contracted and lashes so violently that when the body is attached firmly, it may tear itself loose. The anterior end of the body is thickened to form a collar which bears the heavy spines characteristic of the species. The spines can be seen only under favorable optical conditions and it is very difficult to count them. The acetabulum is situated behind the middle of the body and measures from 0.07 to 0.085 mm. in diameter. Under slight pressure it may be as large as 0.084 to 0.091 mm. in diameter.

The digestive system may be demonstrated clearly by the use of neutral red. The oral sucker is 0.04 to 0.045 mm. in diameter. The pharynx is spherical to oval, about 0.018 mm. in diameter, situated approximately the same distance behind the oral sucker. The esophagus extends almost to the level of the acetabulum and the ceca terminate near the posterior end of the body.

Unicellular glands are abundant throughout the body. In the region between the suckers there are many penetration glands with granular cytoplasm. They are lobed and their number could not be

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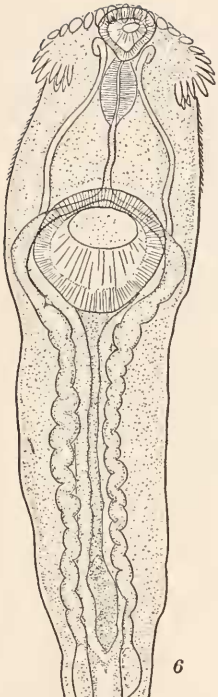
FIG. 6. Immature worm, 3 days in tern; drawn from fixed and stained specimen.

FIG. 7. Immature worm, 12 days in gull; drawn from fixed and stained specimen.

FIG. 8. Adult worm from the intestine of *Larus argentatus*, thirty-one days after cyst was fed; drawn from a fixed and stained specimen.

Plate II



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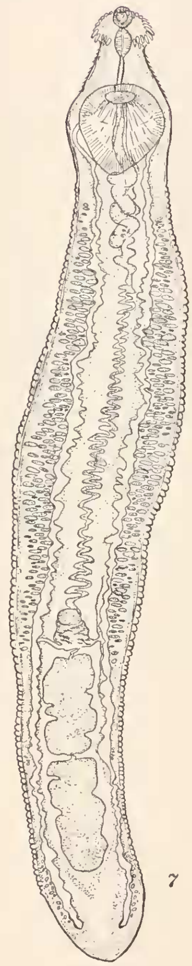

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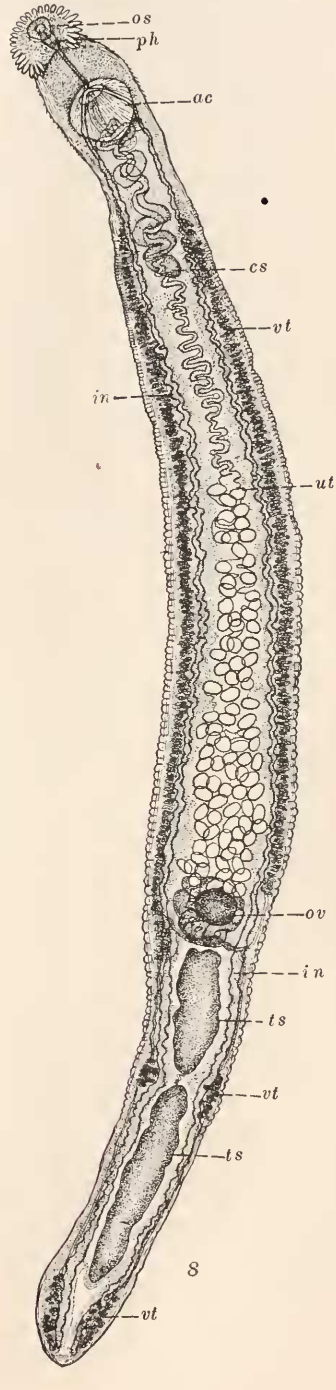

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determined. They do not stain differentially with neutral red. Ducts pass forward on the dorsal side of the body to open at the anterior tip. Much of the body surface on the dorsal side is underlaid with cystogenous glands, in which the secretion occurs in the form of bacilliform rods. Ventrally there are glands which stain intensely with eosin and erythrosin.

The genital anlage consists of a cluster of deeply-staining cells near the caudal end of the body and a row of cells which extends forward to a second cluster near the anterior margin of the acetabulum. The posterior group is the rudiment of the gonads and the anterior one of the copulatory organs.

The excretory system is difficult to trace, because in mature cercariæ the body is so filled with glandular cells, and in immature ones the tissues are so fragile that they will not stand the amount of pressure necessary to demonstrate the tubules and flame cells. There are two excretory pores, one on either side, near the proximal end of the tail. The ducts from the pores unite in the base of the tail and open into a small vesicular bladder in the caudal end of the body. From the bladder, collecting ducts pass forward on each side. These ducts are median to the ceca. They bend mediad in front of the acetabulum and then cross, ventral to the intestinal ceca, to the lateral sides of the body where they continue anteriad to the level of the oral sucker. The collecting ducts contain concretions; those near the ends of the ducts are small, 0.001 to 0.002 mm. in diameter, while those in the region between the suckers are large and measure about 0.005 by 0.009 mm. Near the anterior end of the body the collecting ducts become continuous with the recurrent excretory tubules which pass backward almost to the posterior end of the body. At their caudal ends the recurrent tubules divide to form the secondary and tertiary tubules which lead to the flame cells. The exact arrangement of these tubules and number of flame cells were not determined.

The Metacercaria

Occasionally a cercaria which has been swimming for several hours may encyst in a container or on a slide. Encystment may occur in the presence of irritating substances, e.g., vital stains, which were used to study the penetration and cystogenous glands. Since the cercariæ do not ordinarily encyst in sea water, they were placed in dishes with various animals to discover the intermediate host or hosts in which they pass the next stage of their existence. A cercaria was observed to penetrate into the gill of *Mya arenaria*. It required about forty minutes to enter and encyst. In the process of penetration and encystment, the glands of the cercaria are emptied and a thin-walled cyst is formed. A specimen of *M. arenaria* was placed in a dish with many swimming cercariæ. On dissection of the mollusk four hours later, five cercariæ had lost their tails and were penetrating, one was found in the process of encysting, and twelve metacercariæ were recovered. Penetration and encystment was obtained in the mantle, gills, and foot of *Mya arenaria*, *Modiolus modiolus*, *Mytilus edulis*, *Cumingia tellinoides*, *Pecten irradians*, *Ensis directus*, and *Crepidula fornicata*. Cysts containing metacercariæ (Fig. 5) are spherical to oval, depending on relative pressure at the place of encystment, and measure from 0.14 to 0.19 mm. in diameter.

Development in the Final Host

Experimentally infected specimens of *M. arenaria* were fed to two white rats, three terns, *Sterna hirundo*, and five herring gulls, *Larus argentatus*. The rats did not pass eggs in the feces and both were negative when dissected three and eight weeks, respectively, after feeding. It appears, therefore, that the rat is not a suitable host for the development of the worms. The terns were killed, one day, three days, and twenty days after feeding. The metacercariæ excysted in the intestine of the terns and young worms were recovered from the first two birds examined. The third tern did not pass eggs in the feces and was negative when dissected twenty days after feeding. The gulls were killed, two, three, twelve, thirty-one, and forty-five days after feeding. Large numbers of worms in different stages of development were recovered from the first three gulls, about two hundred gravid worms were found in the fourth gull, and a single worm was present in the intestine of the last one dissected. A young worm removed after one day in the tern measured 0.6 mm. long and 0.154 mm. wide. The spines on the collar were 0.025 mm. in length; the acetabulum measured 0.1 mm. in diameter. The oral sucker was 0.056 mm. in diameter and the pharynx 0.04 mm. long by 0.028 mm. wide. A specimen (Fig. 6), recovered after three days' development in the gull, measured 0.74 mm. in length and 0.23 mm. in width. The collar spines measured 0.0294 mm. in length and 0.0077 mm. in width at the base; the acetabulum measured 0.14 mm. in diameter. The oral sucker was 0.06 mm. in diameter and the pharynx 0.04 mm. long by 0.035 mm. wide. Specimens (Fig. 7), recovered twelve days after they were fed to the gull, measure from 3 to 4 mm. in length. Although they are only about one-half grown, most of them contain eggs in the uterus. These studies have shown that the metacercariæ do not undergo development in the cyst and that the mollusk is actually little more than a transfer host. The metacercariæ were infective for birds three days after encystment and developed to maturity only in the intestine of *Larus argentatus*.

Discussion

Miller and Northup (1926) examined 8,875 specimens of *Nassa obsoleta* from Quamquisset Harbor and found only a single species of echinostome cercaria which they described and named *Cercaria quissetensis*. In the present study over 10,000 specimens of *N. obsoleta* have been examined. All came from the Woods Hole region and about 1,000 of them from the same harbor where Miller and Northup obtained their material. A single species of echinostome cercaria was found which is so similar to that described by Miller and Northup that the two must be regarded as identical. The measurements agree closely and the differences between the descriptions are readily explainable. Miller and Northup reported only 27 cephalic spines. In the present study the exact number, 31, was first counted in the metacercaria and later confirmed in the cercaria.

In the preliminary papers (Stunkard 1934*a*, 1937), the adult worms recovered from the herring gull were regarded as specifically identical with those described by Linton (1928) from four species of Larus and from *Nycticorax nycticorax* and identified by him as *Himasthla elongata* (Mehlis). Although he referred his specimens to *H. elongata*, Linton noted certain differences between them and previous descriptions of that species. Linton's determination was questioned by Vogel (1933) and comparison of the present worms with the description of *Himasthla elongata* as given by Dietz (1910) shows such marked differences that they cannot be included in the same species. There are constant differences in the number and arrangement of the cephalic spines, size of organs, shape of testes, and in distribution of the vitellaria. In *H. elongata* the vitelline follicles are continuous, whereas in the species which I have studied these glands are interrupted at the levels of the testes. Since this species is distinct from *H. elongata*, the name given by Miller and Northup to its cercarial stage was adopted. The specimens identified as *H. elongata* by Linton were accordingly referred to *H. quissetensis*.

In the paper cited, Linton (1928) reported on several hundred specimens of *Himasthla* which had been collected at Woods Hole, Massachusetts, from four species of *Larus* and from *N. nycticorax*. He stated, "These distomes, while they vary considerably in size and proportions, appear to belong to the same species, and are in such close agreement with *H. elongata* that it seems best to refer them to that species in spite of the difference in the number of circum-oral spines.

H. elongata is characterized by having 29 circum-oral spines, of which the two which are situated at each angle of the oral disc are smaller than the others. In all of the specimens in which they could be distinctly seen in the Woods Hole material, the number of oral spines was found to be 31, arranged as shown in Figure 18." In this figure, 27 spines are situated in a single row with two between and behind the others at the ends of the row.

In addition to the specimens referred to *H. elongata*, Linton described two specimens from the white-winged scoter, *Oidemia deglandi*, as a new species which he named *Himasthla incisa*. In this species he reported that there are about 27 cephalic spines. The type specimen of *H. incisa* and representative specimens of the worms referred to *H. elongata* were deposited in the United States National Museum.

Lack of agreement between the number of cephalic spines on specimens referred by Linton to *H. elongata* and the number previously reported for that species, together with differences in the arrangement of the vitellaria in Linton's specimens and those of *H. quissetensis* have created a problem of specific determination. For comparison with material of *H. quissetensis*, Linton's specimens were borrowed from the U. S. National Museum. There were five slides, numbered 7921 to 7925 inclusive. The type specimen of *H. incisa* was mounted on slide No. 7925. This worm has 31 cephalic spines but the anterior part of the specimen is twisted and flattened, so their arrangement at the ends of the row is not symmetrical. The worm has heavy muscles and the cuticula bears prominent spines which extend posteriad as far as the level of the testes. The vitellaria are massive and extend along the sides of the body without interruption. Other specific features are presented in the measurements and figure of Linton. The specimen is clearly distinct from *H. quissetensis* and from all other described species. All of the other specimens had been identified as *H. elongata* by Linton. A single specimen from *Larus marinus* was mounted on slide 7923. It has 31 cephalic spines and the vitellaria are interrupted at the testicular zones. In both of these features as well as in total size and in the position and size of the individual organs it agrees with *H. quissetensis*. Consequently, I believe that this specimen should be assigned to that species. Nine specimens from *Larus argentatus*, which obviously belong to the same species, are mounted on slide 7921. They agree with Linton's figure 17 and all of them have 29 cephalic spines, 25 arranged in a single row with two between and behind the others at the tips of the collar. It is apparent that these specimens are distinct from the two described previously and their systematic position is doubtful. They may belong to *H. elongata* as Linton believed. A single specimen from *Nycticorax nycticorax* is mounted on slide 7924. It bears 29 cephalic spines and is probably identical with the worms from *L. argentatus* on slide 7921. Three specimens from *Larus delawarensis* are mounted on slide 7922. They have 29 cephalic spines, and although they are immature, the worms agree structurally with those on slides 7921 and 7924.

Subsequently, Professor Linton kindly sent additional material from his own collection. In a personal communication (April 11, 1938) he wrote, "Upon looking over my slides I find that I must have included a lot from the Herring Gull in my former report that were not *H. elongata*, as I find only 11, instead of 34 slides of that species." Among the specimens received from Professor Linton there is one which has 31 cephalic spines and interrupted vitellaria and which I regard as *H. quissetensis*. The others have 29 cephalic spines and are identical with the specimens on slides 7921 and 7924 from the U. S. National Museum collection.

It appears, therefore, that the specimens identified by Linton as *H. elongata* belong to two distinct species. Most of the worms agree with his figure 17 and they may be *H. elongata*. They have 29 cephalic spines and the measurements of twelve representative specimens are intermediate between or overlap the figures given by Dietz (1910) as characteristic for *H. elongata* and *H. militaris*. In my opinion the worms might with equal justification be referred to either of the two species. On the other hand, they may belong to neither. The second group of specimens referred by Linton to *H. elongata* have 31 cephalic spines and agree with the worms which I have described as *H. quissetensis*. The anterior end and cephalic spines of one of these specimens is shown in Linton's figure 18.

The precise status of the present and other species which have been referred to the genus *Himasthla* is doubtful. Until the amount of variation which naturally occurs in a species is determined, and its limits defined, final specific determination is impossible. In a related species, *Echinostoma revolutum*, Beaver (1937) has studied the amount and nature of variation. Certain of his observations are pertinent to the problem at hand. He found, p. 19, "The range then for mature worms is from 4 to 30 mm. in length. This size range is so great that a description of any one size is grossly inadequate for an accurate diagnosis of all other sizes, and a description by the ordinary methods is impractical when applied to worms having so great a range in size. This would not be true, however, if it were possible to give proportionate measurements applicable to all sizes which, as shown below, cannot be done. Practically every feature has been measured and plotted; and in no instance is the proportion between two organs or structures a constant throughout the series." He stated, p. 26, "Egg size varies with the age of the worm and is possibly somewhat altered by the host. The younger worms produce eggs with a very great range in size, and the average length is much greater than in more mature ones. The range in size for the eggs of the worms from all hosts was found to be 91 to 145 microns by 66 to 83 microns." Among other conclusions he reported, p. 65, "It is shown by this study that the cephalic spination is the most reliable character for diagnosis of the adult worm."

Sprehn (1932) listed *H. militaris* (Rudolphi, 1803) and *H. secunda* (Nicoll, 1906) as synonyms of *H. leptosoma* (Creplin, 1829) and Palombi (1934) admitted the probable identity of *H. leptosoma* and *H. secunda*. Since three of the four species of *Himasthla* which bear 29 cephalic spines were regarded as identical by Sprehn, one is led to question why the other one, *H. elongata* (Mehlis, 1831) was accepted as valid. There appears to be no better reason for retaining *H. elongata* than the other specific names which were dropped in synonymy. If the opinion of Sprehn is correct, the name of the species is *H. militaris* and not *H. leptosoma*.

With reference to *H. quissetensis*, it is not impossible that this species is identical with *H. alincia* Dietz, 1909, which also has 31 cephalic spines. The description of the latter species is based on a single specimen from *Tringa cinclus*, collected in Brazil. The figure of Dietz suggests the appearance of a worm which was dead before fixation and consequently much elongated. The worm is much more extended than the specimens which I have studied but the only obvious morphological difference between them is in the anterior limits of the vitellaria, which in *H. alincia* do not extend to the level of the cirrus sac.

A further case of specific identity is suggested between *H. quissetensis* and *H. muehlensi*, the species described by Vogel (1933) from man. A comparison of the specimens at hand with the description of *H. muehlensi* shows much similarity especially in the distribution of the vitellaria. The worms studied by Vogel were slightly larger and apparently contained one more spine in the cephalic coronet. But those specimens were dead and somewhat macerated; in none of them was the crown of spines complete and intact, and the absence of lateral crenations in the body wall may be correlated with the greater length of the extended specimens. Indeed, a specimen of *H. quissetensis* which was fixed in a moribund condition, is much extended and measures 14.2 mm. in length. The tissues had absorbed water and all the organs are larger than corresponding ones in well-fixed specimens. The testes were almost smooth. The differences between *H. quissetensis* and *H. muehlensi* in size and in dimensions of particular organs are not surprising in view of the difference in hosts and condition of material. Indeed, Vogel noted that species of *Himasthla* had previously been reported only from birds and he raised the question whether *H. muehlensi* may not normally be a bird parasite which occasionally is introduced into the human intestine where it may become mature but in which it is probably retained for only a short time. Consideration of this problem and of the possible difference in number of spines is facilitated by certain observations of Beaver (1937). He found in *Echinostoma revolutum* that large individuals may produce accessory collar spines. He stated that ordinarily the worms do not attain a definitive size, but continue to grow until they are expelled. From the study of specimens developed in six different mammalian and three different avian hosts he reported, p. 28, "As a rule the worms develop more rapidly in birds (Table 7, p. 77), become mature earlier and at a smaller size, and live a much shorter period." The data of Beaver show that differences induced in individuals of the same species when reared in avian and mammalian hosts are as great as those between *H. quissetensis* and *H. muehlensi.*

The first experimental demonstration of the life cycle in the genus *Himasthla* supports the opinion of Vogel that the human species, *H. muehlensi*, is acquired by eating raw or insufficiently cooked mollusks. Examination of large numbers of *Venus mercenaria*, purchased in the New York market, has failed, however, to disclose the metacercaria of this species. Whether or not it is distinct from *H. quissetensis* remains to be determined.

Results of the present study on *H. quissetensis* supplement those of Beaver on *Echinostoma revolutum*. All of the gulls were fed approximately the same number of metacercariæ and 100 to 200 worms were recovered from the intestine of each of the birds killed two, three, twelve and thirty-one days after experimental feeding. The worms collected after thirty-one days in the intestine may not have been fully mature, since the terminal half of the uterus contained very few eggs. The bird sacrificed forty-five days after ingestion of cysts had been passing large numbers of eggs in the feces but contained only a single specimen when killed. It appears that in the present species the worms do not persist in the intestine very long after they become sexually mature.

Summary

Cercaria quissetensis Miller and Northup, 1926 has been restudied. It is the only echinostome cercaria which has been found in the

examination of over 18,000 specimens of *Nassa obsoleta* at Woods Hole. This cercaria encysts in the gills, mantle and foot of various mollusks. Metacercariæ were obtained experimentally in *Mya arenaria*, *Modiolus modiolus*, *Mytilus edulis*, *Cumingia tellinoides*, *Pecten irradians*, *Ensis directus*, and *Crepidula fornicata*. The metacercariæ are infective for birds three days after encystment and develop to maturity in the herring gull, *Larus argentatus*. Sexually mature specimens are identical with certain of those described by Linton (1928) as *Himasthla elongata* (Mehlis). Since there are important differences between the present specimens and *H. elongata*, they cannot be referred to that species and the name of the cercaria is adopted for them. Possible synonymy in the genus *Himasthla* is discussed.

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